



BNC105 induces tumor micro-environment changes which enhance the efficacy of checkpoint inhibitor therapy in preclinical models

Daniel J Inglis, Donna M Beaumont, Annabell F Leske, Michaela A Scherer, Tina C Lavranos

Bionomics Ltd, Adelaide, Australia

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BNC105 creates an immunogenic environment

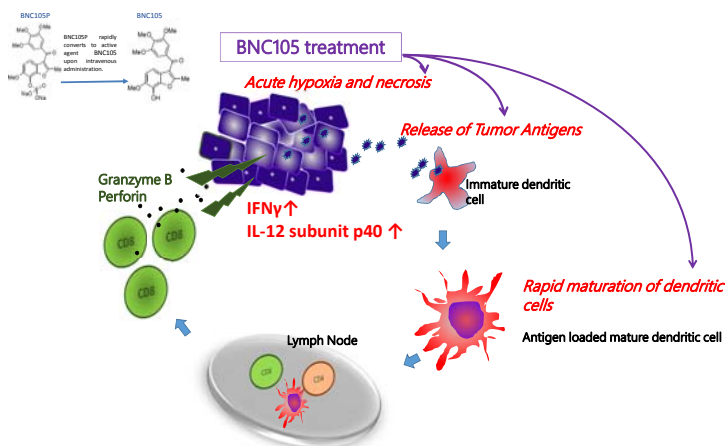
BNC105 is a Phase II tubulin depolymerisation agent that exerts direct anti-cancer efficacy through the selective destruction of solid tumor microvasculature and direct suppression of tumor cell proliferation. A single i.v. dose of BNC105 causes a very high degree of tumor hypoxia leading to >95% necrosis in rodent tumor models. The therapeutic window of BNC105 has been shown to be superior when compared to similar compounds in its class, highly specific to tumor vasculature with minimal off target activity, proving it to be clinically well tolerated. Complementary studies in models of chronic lymphocytic leukaemia have shown that BNC105 also activates JNK dependent apoptosis, mediating cancer cell death.

Currently there is a very strong drive to extend the clinical benefit of immunotherapies to a broader patient population where many patients fail to respond. This translates to a strong focus being placed on 'awakening' tumors and stimulating the immune system to become reactive to tumors that would otherwise be tolerated and evade the unequivocal therapeutic benefit of checkpoint inhibitors.

In the absence of an innate and pre-existing tumor immune response, stimulating an initial immune response by altering the immune homeostasis can be the key to making immunotherapy relevant to more patients. The placement of BNC105 in this therapeutic setting is potentially beneficial for sustainable patient response to immunotherapy.

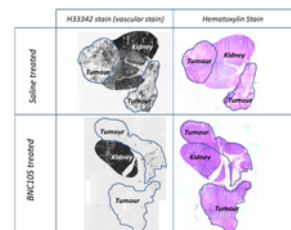
BNC105 induced tumor microvasculature destruction, tumor hypoxia and necrosis led to changes in the tumor microenvironment and the surrounding immune landscape. Recent evidence demonstrates that microtubule-depolymerizing agents not only cause an efflux of immune presenting tumor antigens via tumor disruption/necrosis but also enhanced maturation of dendritic cells into antigen presenting cells and the release of pro and anti-inflammatory cytokines. This has a net effect of firmly disrupting the *status quo* of immune dormant tumors and is an opportunity to re-invigorate anti-tumor immunity. These observations led us to examine key immune clinical biomarkers from BNC105 treated patients and investigate pre-clinically the potential therapeutic benefit of combining BNC105 with the checkpoint inhibitors that target PD-1 or CTLA-4.

BNC105 disrupts the immune homeostasis of the tumor micro-environment priming the tumor for a sustainable response to immunotherapy



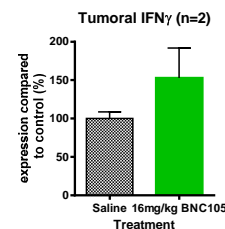
BNC105 causes acute tumor damage and increases tumor IFN γ

BNC105 causes rapid destruction of tumor vasculature and is highly selective leaving normal vasculature intact. In a murine orthotopic model of Renal Cancer (Renca) we have shown using perfusion of a vascular stain that tumor blood vessels are obliterated after BNC105 treatment compared to normal tissue which remains unaffected.



Perfusion of the vascular stain H33342 into RENCA (mouse renal adenocarcinoma) orthotopic tumors 4 hours post a single dose of BNC105 i.v.

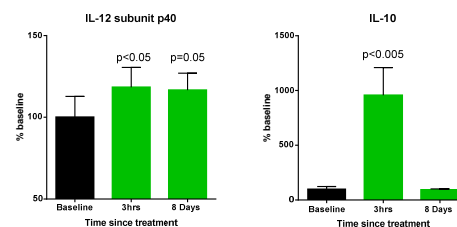
BNC105 primes the immunogenic potential of a tumor with increased tumoral IFN γ content compared to control treated animals. IFN γ is secreted from CD4+ Th1, CD8+ and Th0 cells and activated NK cells. No change in tumoral CD3+/CD8+ cells was seen suggesting an increase in the component of complementary immune cells fostering an environment for tumor specific immune activation when checkpoint inhibitors are deployed.



BNC105 clinically enhances the immune response IL-12 p40 and IL-10

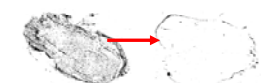
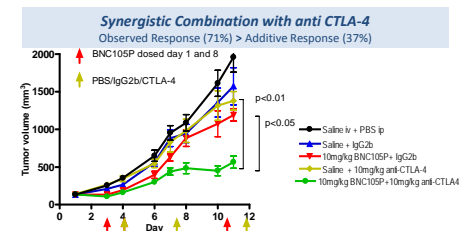
The balance between pro-inflammatory and anti-inflammatory signals provided by different immune cell populations is crucial for normal physiology and the suppression of cancer development. By altering this homeostasis an opportunity is provided for the immune system to alter the way it responds. Biomarker analysis on patient samples was conducted from a Phase II BNC105 monotherapy mesothelioma trial. Biomarker analysis showed that plasma IL-12 subunit p40 significantly increases post-BNC105 administration and remains elevated at Day 8 post dosing. The immune-modulatory cytokine IL-12 subunit p40, a key member of the IL-12 cytokine family, has emerged as a potent inducer of antitumor immunity. IL-12 subunit p40 is secreted by activated macrophages that serves as an essential inducer of Th1 cells development. This cytokine has been found to be important for sustaining a sufficient number of memory/effector Th1 cells.

Significant changes were also seen in levels of the immune-modulatory cytokine IL-10. IL-10 mediated stimulation of adaptive immunity to tumors has been observed clinically. IL-10 increases monocytes which are able to induce the expansion of tumor resident CD8+ T cells in tumors and enhance their cytotoxic activity.



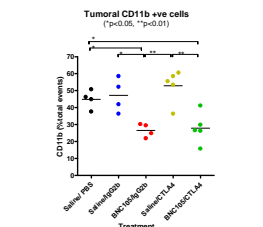
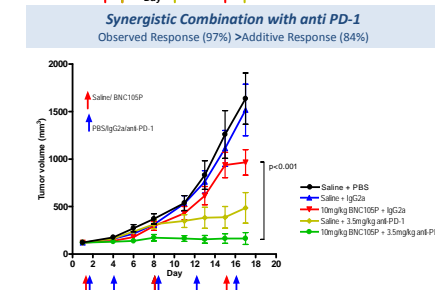
Phase II Mesothelioma trial BNC105 (16mg/m²) number of patients >19. Blood draws were pre-specified and optional. Patients receiving BNC105 alone received blood draws prior to BNC105 administration and 3 hours following administration. Plasma samples were used to determine exploratory analytes using Multi-Analyte Profile (MAP) technology (Myriad RBM). Graph showing % change from baseline. Percent change was calculated as analyte plasma concentration (post - baseline) / baseline * 100. Mean ± SEM shown on graph.

Anti CTLA-4/PD-1 efficacy with BNC105 in murine colon cancer model



CT26 tumor Vascular perfusion before/after BNC105 treatment
The perfusion of the vascular stain H33342 into CT26 tumors in balb/c mice demonstrates under fluorescent microscopy the highly effective disruption of tumor vasculature by a single dose of 10 mg/kg BNC105 i.v.

A significant reduction in the number of tumor infiltrating macrophages (CD11b+) was seen after treatment with BNC105 (monotherapy and combination). This reduction would dramatically change the immune environment potentially releasing the immune dampening effect of macrophage subsets.



Summary

The immune activation that results from changes to the tumor and its micro-environment is a key opportunity to leverage a response from checkpoint inhibitors in tumors that would otherwise be tolerated by the immune system. This mechanism would allow for the immense therapeutic value of checkpoint inhibitors to push deeper into patient populations generating a larger number of 'responders'.

BNC105 is a Phase II potent and highly selective disruptor of tumor microvasculature causing rapid onset of tumor hypoxia and necrosis. Tubulin depolymerising agents can cause an efflux of tumor antigens being presented to the immune system and can additionally accelerate the maturation of dendritic cells rapidly generating antigen presenting cells.

It was shown pre-clinically that tumoral IFN γ , a key regulatory cytokine, is induced by BNC105 treatment correlating with an influx of complementary immune cells. Additionally CD11b+ cells were significantly reduced after BNC105 treatment, subsets of which are known to repress immune activity. This priming of the immunogenic potential of a tumor may work directly in concert with checkpoint inhibitors. The strong synergy of BNC105 with both anti CTLA-4 and PD-1 was demonstrated in immune-competent *in vivo* models with high tumor growth inhibition observed. This synergy may be due to BNC105 priming the immune system, complementing the action of the checkpoint inhibitors.

Clinically BNC105 has been shown to increase plasma IL-10, a known inducer of tumoral CD8+ cells which also enhances their cytotoxic activity via elevated granzyme release. IL-12 subunit p40, the pro-inflammatory cytokine, shown pre-clinically to be a potent and robust inducer of anti tumor immunity was also significantly increased by BNC105 treatment in clinical patient samples. Rapidly shifting the balance of pro-inflammatory and anti-inflammatory signals provides an opportunity for an elevated immune response towards the tumor.

These findings strongly support clinical evaluation of BNC105 in combination with checkpoint inhibitors. BNC105 driven priming of the tumor and immune system may extend the reach of checkpoint inhibitors to leverage a therapeutic benefit to a greater patient population.

Synergy defined to occur when the Observed Response (R_{obs}) > Additive Response (R_{add}). $R_{add} = \text{Fraction (F)}_{BNC105} + \text{Fraction (F)}_{CTLA-4} + \text{Fraction (F)}_{PD-1} \times 100\%$